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NUTRITIONAL MILESTONES IN THE ANEMIAS

The Macrocytic Anemias.

The search for a nutritional anti-anemia factor has occupied the attention of investigators in various fields of the biological sciences for more than two decades. The problem has been complicated not only by the elusive nature of the hypothetical factor involved, but also by the complexity and diversity of the various anemias. There are a number of forms of anemia which, thus far, have eluded control by any nutritional means. On the other hand, the dietary management of the simple anemia of iron deficiency is a well established success. The control of the various macrocytic anemias with liver extract and, more recently, with folic acid, marks another victory in the fight against this disease.

The Anemias

The anemias are classified into numerous types based primarily on the structural pattern of the blood changes and the nature of the factors inducing these changes. The structural changes are reflected in the number, size and hemoglobin content of the red blood cells or erythrocytes. Concomitant with these are usually changes in the white blood cells, leukocytes, as well.

Normocythemia implies a normal erythrocyte count; *hypocythemia,* a decreased count; *hypercythemia* or *polycythemia,* an abnormally increased count. Erythrocytes which are normal in size are described as *normocytic;* decreased in size, *microcytic;* increased in size, *macrocytic.* When the erythrocytes contain the normal amount of hemoglobin, they are described as *normochromic,* when the amount of hemoglobin per cell is decreased, they are called *hypochromic;* when it is increased, they are *hyperchromic.*

The anemias of iron deficiency, for example, are, described as *microcytic, hypochromic anemias.* This indicates that the red blood cells are subnormal in size and hemoglobin content. Their total number is generally reduced as well.

The anemias to be discussed in this Review are, on the other hand, all of the *macrocytic, hyperchromic* type. In this type the red blood cells are greatly reduced in number, the individual cells, however, being abnormally large and containing more hemoglobin, per cell, than do normal cells. (This is apparently an attempt to compensate for the severely decreased number of red blood cells.) The net effect, even though the cells are large and contain much hemoglobin, is a striking deficiency of total hemoglobin in the blood.

The primary function of the erythrocytes and of hemoglobin is the transportation of oxygen from the lungs to the tissues where it is vitally needed for the oxidative processes of cell metabolism. Consequently, any combination of the above pathological changes having a net effect of reducing the amount of hemoglobin in the circulating blood will cause a corresponding reduction in the oxygen-carrying power of the blood. This in turn deprives the various organs and tissues of sufficient oxygen (anoxia) leading to a number of symptoms characteristic of all types of anemias. The symptoms may include pallor; generalized muscular weakness with lassitude and easy fatigue; disturbances of the gastrointestinal tract due to loss of muscle tone resulting from the anoxia—loss of appetite, nausea, constipation, gaseous distention; and increased heart rate and blood flow to compensate for the diminished oxygen-carrying power of the blood, often resulting in palpitation and shortness of breath.

The causes of anemia are many and are not all clearly understood. The present Review will be limited to the group of so-called macrocytic, hyperchromic anemias related to the lack of one or more nutritional factors*. These include pernicious anemia, nutritional anemia, and the macrocytic anemia of sprue, pellagra and pregnancy.

The Nutritional Factor:

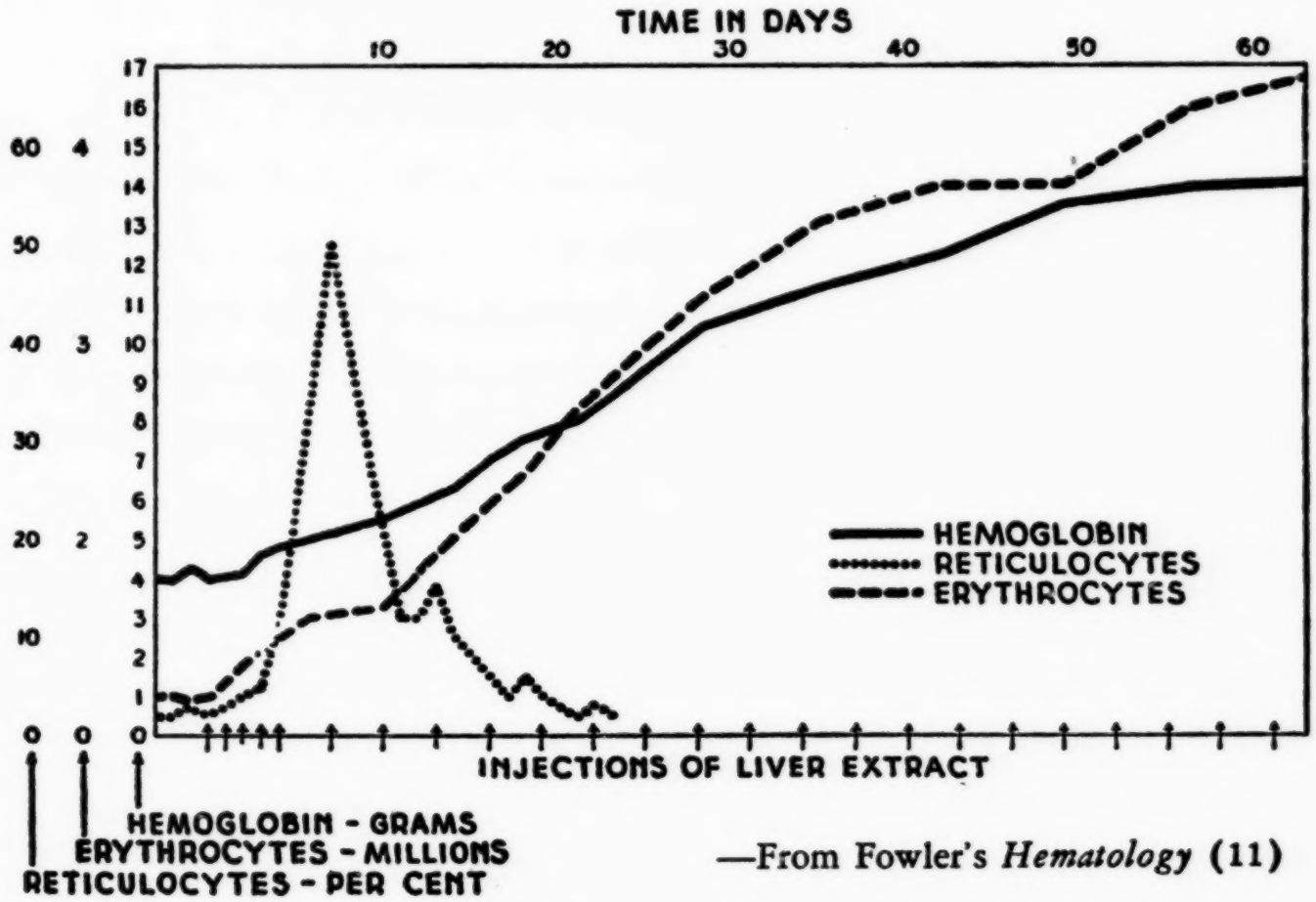
The search for a nutritional factor in the etiology of pernicious anemia and of the other closely related macrocytic anemias is a long and exciting story which had its first dramatic success twenty years ago. Following Whipple's experiments on blood regeneration in dogs made anemic by repeated bleedings and cured by the feeding of liver, Minot and Murphy (1) demonstrated the similar effectiveness of liver therapy in the management of human pernicious anemia. Three years later Castle (2) set forth his well-known theory of the "intrinsic" and "extrinsic" factors in anemia. These factors, the one present in normal gastric secretions and the other in certain foods, of which liver is an outstanding example, interact to form a substance which is essential for the development of mature erythrocytes. This substance is known as the erythrocyte maturing factor (E.M.F.). This factor, when supplied to anemic patients, causes marked remission of the symptoms of pernicious anemia. The E.M.F. may be formed within the body, possible sites of formation being the intestinal tract or the liver, or it may occur preformed in liver as an active anti-pernicious principle. That the E.M.F. and the so-called anti-pernicious anemia factor of liver are one and the same is strongly suspected (2a), although a slight difference in their

*This group does not include the microcytic, hypochromic anemia of iron deficiency which has been discussed in an earlier Review (April, 1944).

chemical properties suggests that perhaps the erythrocyte maturing factor may be a precursor of, rather than identical with, the anti-pernicious anemia factor.

The identity of these factors has been a matter of conjecture for the past two decades. Of particular interest to nutritionists has been the long search for the extrinsic factor in food. While conclusive evidence is not yet at hand, the near future may reveal the close relationship of the new B vitamin, folic acid†, to the anti-pernicious anemia factor, the erythrocyte maturing factor or its components.

PERNICIOUS ANEMIA
Reticulocyte Response to Liver Therapy



—From Fowler's *Hematology* (11)

FIG. 1—The response of the reticulocytes, erythrocytes, and hemoglobin to liver extract in a patient with pernicious anemia.

Soon after the pioneer work of Minot and Castle and their associates, Lucy Wills (3) who was engaged in an inquiry into the causes of maternal mortality in Bombay, India, reported the successful treatment of tropical macrocytic anemia with yeast concentrates. This type of anemia, frequently a severe and fatal complication of pregnancy in tropical climates presented a blood picture identical with that of pernicious anemia. This suggested at first the therapeutic trial of liver extracts.

†*cf.*—*Borden's Review of Nutrition Research,* February, 1946—"Folic Acid."

> Because of the identical blood picture seen in pernicious anemia and in the various macrocytic anemias of nutritional origin (anemia associated with simple dietary deficiency, with pregnancy, sprue and pellagra) it had long been supposed that the two types of anemia were identical. Recent studies have, however, demonstrated a distinction between pernicious anemia and the other macrocytic anemias with respect to their causes, behavior and relationship to dietary factors.

The treatment met with great success. The success of liver therapy, combined with the observation of spontaneous remissions after pregnancy when the nutritional demands of the fetus were removed, led to the conclusion that the anemia was in large part a deficiency disease. Laboratory experiments with monkeys (4) further confirmed this theory when it was found that the animals developed a severe anemia on a typical native diet. The symptoms were relieved by supplements of yeast extract, leading the investigators to postulate some member of the vitamin B complex as the factor involved. The substitution of yeast extract for liver extract in their human patients led to a gratifying remission of symptoms thereby leading Wills to conclude that yeast extract contained a curative agent for macrocytic anemia equally as potent as liver extract. The possible common identity of the factors in the two materials was strongly considered.

A few years later in 1935, Day and associates (5) in the United States reported an identical anemia syndrome in monkeys on another type of deficient diet. Again, the symptoms responded to yeast extract. The unidentified anti-anemia factor in yeast was named "vitamin M."

There then followed a fascinating series of independent animal studies on chicks, microorganisms, rats and other species described in the February issue of this Review. Slowly, but with dramatically increasing momentum, the accumulated evidence in all of these fields of animal investigation finally converged to reveal the active factor(s) to be one of the number of forms of the *L. casei* factor, popularly known as "folic acid."

Human Studies:

Nutritional Macrocytic Anemia: The occurrence of a macrocytic anemia in patients with pellagra or other vitamin B complex deficiency states has been known for some time, but it was not until a little more than a year ago that detailed study was given to this problem and some of the long-standing questions involved, partially answered. At that time, Moore, Vilter, Minnich and Spies (6) published an account of their clinical experience over a period of years with this type of anemia, cytologically indistinguishable from pernicious anemia.

Twenty-five patients were selected for study from a total of 56 patients with macrocytic anemia observed in the Nutrition Clinic of the Hillman Hospital from 1940 to 1943. Careful compilation of diet histories and detailed diet analyses disclosed that all of the patients had been deficient in animal protein and the B complex for years. Estimation of the amount of Castle's extrinsic factor in their diets was difficult because few data exist on the extrinsic factor activity of various foods. Known sources of this factor include beef muscle, milk, eggs, liver, yeast, rice polishings and wheat germ, all also excellent sources of the vitamin B complex. Since evidence existed to show that the extrinsic factor is usually associated with the B vitamins, it was assumed that the patients who exhibited clinical evidence of vitamin B deficiencies also were deficient in the extrinsic factor. This assumption was indeed confirmed when several of these patients were given daily for a test period an amount of ground beef muscle which had been shown by Castle *et al* (2, 7) to contain enough extrinsic factor to induce remissions in pernicious anemia patients when fed in combination with normal gastric juice. The vitamin-deficient patients responded to feedings of beef muscle with significant rises in the reticulocyte count. This, however, was not followed by any appreciable increase in hemoglobin or red blood cells and was attributed to the fact that the various concomitant vitamin B deficiency symptoms (diarrhea, inflammation of the tongue, etc.) interfered with both eating and with proper absorption from the gastrointestinal tract, thereby minimizing the amount of extrinsic factor available from the diet.

The phenomenon of reticulocytosis (an increase in number of reticulocytes in the blood stream) has been observed to be a major, typical response to liver therapy in pernicious anemia as well as in the various related macrocytic anemias (8, 9, 10). The exact nature and function of the reticulocyte (reticular or net-like cell) is not clearly understood. It is believed to be a structural variation of the normal erythrocyte and is generally thought to represent a stage in the maturation of the red blood cell (11). For this reason, an increase in reticulocytes is usually accepted as an indication of active erythropoeisis (red blood cell formation) even before any increase in hemoglobin or erythrocytes is noted.

Control of these symptoms with niacin and other B vitamins in a subsequent experiment permitted not only reticulocytosis following the feeding of beef extract, but also the expected subsequent rise in erythrocyte value.*

The very fact that these individuals responded to a dietary source of the extrinsic factor was evidence that the gastric glands were secreting sufficient intrinsic factor for the formation of the essential erythrocyte maturing factor. This was further demonstrated by means of an unusual biological assay

*The erythrocyte increase was, however, of a rather low order, indicating that manufacture of the intrinsic factor was proceeding at a reduced rate.

experiment in which beef muscle or its extract (sources of extrinsic factor) was incubated with the gastric juice of pellagrins suffering from macrocytic anemia and fed to pernicious anemia patients. A typical reticulocyte response was obtained, indicating that the gastric juice of the pellagrous patients contained the intrinsic factor (missing in the pernicious anemia patients) necessary to form with the beef factor the erythrocyte maturing factor required by the pernicious anemia patients.

The nature of the extrinsic factor, however, still remained a mystery. The patients responded, as already indicated, to beef muscle or its extracts and also to highly purified liver extracts. Administration of the various B complex vitamins, nicotinic acid, thiamine, riboflavin, calcium pantothenate, pyridoxine, inositol, para-aminobenzoic acid and choline, singly or in combination, while effective in controlling the symptoms of pellagra produced no hematological response whatsoever.

The dietary management of this type of anemia was followed by marked improvement and, where adequate diets were continued after leaving the hospital, patients remained well and required no further therapy. (In pernicious anemia, on the other hand, lifetime liver therapy is essential to provide not only the dietary factor, but chiefly the intrinsic factor which the anemia patient is unable to produce). The authors have summarized the clinical course of the nutritional macrocytic anemia of pellagra as follows:

> "Coincident with the development of a hematologic remission, induced either by beef muscle or liver extract, the patients showed subjective improvement. Their appetites improved, they regained their strength, and they were able to resume their normal activities. After liver extract had been given, the diarrhea stopped. In the majority of instances, patients felt better and were more energetic than they had been for years. . . . Seven of the twenty-five patients corrected their faulty dietary habits and began eating normal amounts of animal protein. They have remained well and free of diarrhea and the anemia has not reappeared during the two or three years which have since elapsed, even though no liver therapy has been given during this time. They were not given special low fat diets. The other patients who returned to their inadequate food habits developed recurrences of the diarrhea, anemia, and other manifestations after eighteen to twenty-four months; they required further therapy."

Related Macrocytic Anemias: As has already been mentioned, a number of other types of anemia have been studied which, from blood and bone marrow examination, appear to be identical with each other and with the macrocytic anemia of pellagra. These include pernicious anemia, tropical macrocytic anemia, the macrocytic anemia of pregnancy (distinct from nutritional iron-deficiency anemia of pregnancy) and the macrocytic anemia of sprue. The

differential diagnosis of these macrocytic anemias has been very difficult and, it is not unlikely that many cases of nutritional macrocytic anemia, for example, have been erroneously diagnosed as pernicious anemia.

A number of criteria exist for distinguishing pernicious anemia from the other macrocytic anemias, the chief one being the absence of free hydrochloric acid in the stomach of pernicious anemia patients. Failure to respond to extrinsic factor alone is another indication of pernicious anemia. Even these criteria, however, are not infallible since achlorhydria may be a temporary occurrence in nutritional macrocytic anemia (6) and the extrinsic factor if given in massive doses may even stimulate a response in pernicious anemia (12). Differentiation between nutritional macrocytic anemia and the anemia of sprue is even more difficult, if indeed the two anemias are distinct. In both instances, the anemia is caused by poor intake of extrinsic factor, poor absorption of the erythrocyte maturing factor, inadequate production of the intrinsic factor, or a combination of these influences (6). The same problems exist in determining the etiology of the defects in tropical macrocytic anemia which, although manifested by both sexes, occurs predominantly during pregnancy. As Moore and associates (6) point out:

> "The practical importance of making the differentiation *(between nutritional macrocytic anemias and pericious anemia)* is evident when one considers that extrinsic factor deficiencies can be corrected by dietary means alone and do not require lifelong administration of liver extract."

It has been suggested that all of these anemias may, as a matter of fact, have an ultimate common origin and as one writer (13) has pointed out:

> "It seems probable that if studies were comprehensive enough all grades of deficiency would be found, from 'pernicious anemia' where intrinsic factor deficiency plays the major role on the one hand, to the cases of pellagra where dietary lack is perhaps the chief factor, at the other extreme."

At any rate, the final mechanism for producing extrinsic factor deficiency anemia and intrinsic factor deficiency anemia (pernicious anemia) appears to be the same as that causing the nutritional macrocytic anemias: namely, an insufficiency of the erythrocyte maturing factor. This may result either from inherent defects such as impaired gastric function (intrinsic factor failure) or from a failure to eat an adequate diet (extrinsic factor deficiency.)

The studies of Moore, Vilter, Minnich and Spies (6), however, have at least cleared up a great many of the questions concerning the nature and causes of the nutritional macrocytic anemias. They have summarized their conclusions as follows:

"The nutritional macrocytic anemia of pellagra apparently depends upon the existence of a prolonged dietary deficiency of extrinsic factor associated in many instances with poor absorption from the intestinal tract and/or inadequate production of intrinsic factor by the gastric mucosa. Final proof cannot be obtained until pure extrinsic factor has been isolated and its effect tested on patients with similar anemia."

In October, 1945 an excellent editorial in the British Medical Journal (14) reviewed and discussed the subject of nutritional macrocytic anemia. Remarking that the long-standing question of the pathogenesis of this type of anemia has now been decisively answered by the studies of Moore *et al*, the editorial acknowledged the excellence of this research as a challenge to British hematology, pointing out that the British Empire is the world's largest reservoir of nutritional macrocytic anemia. The editorial concluded with a plea for the setting up of specific research activities which might lead to the discovery of a cheap remedy for this widely prevalent disease which, if found, would greatly enhance the vigor of their immense tropical and subtropical populations. As this editorial was being written, the Southern Medical Journal in the United States was setting into type for its forthcoming November issue, a new report from the Birmingham Nutrition Clinic. The report was entitled: "Observations of the Anti-Anemic Properties of Synthetic Folic Acid." From all indications, it appeared that folic acid was the urgently sought object of the British quest for a nutritional factor in the control of the macrocytic anemias.

The April issue of *Borden's Review of Nutrition Research* will describe the results of clinical studies with folic acid.

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NEWS DIGEST

"NEW" 80% FLOUR — Effective on March 1st, millers are required to increase the wheat flour extraction percentage as part of a national measure to extend the supply of wheat for consumption at home and abroad. In customary milling processes, 100 pounds of cleaned wheat yield about 72 pounds of flour, known as *straight flour.* The remaining 28 pounds of wheat, consisting chiefly of branny parts and germ, are used primarily for livestock feed. The removal of from 4 to 8 additional pounds of the lower quality flour from the *straight flour* yields the superior quality creamy colored baking flour known as *patent flour.* Thus about 64 to 68 pounds of *patent flour* are ordinarily obtained from an original 100 pounds of cleaned wheat. Under the new order, millers are required to make 80 pounds of *straight flour* (instead of 72 pounds) from each 100 pounds of cleaned wheat. An additional 8 pounds of the slightly darker parts of the wheat kernel must be retained in the straight flour. Bread made from the "new" flour is slightly creamier in color than the usual white bread. The change in taste is said to be barely perceptible. Reports are not yet available on the baking qualities of the new 80% extraction flour. *Practical Home Economics 24,* 178 (1946).

TENDERIZED FROZEN MEAT — Recent research has shown that the faster meat is frozen the greater the tenderness it acquires. For example, beef frozen at 18° F. and then thawed proved to be about 9 per cent more tender than unfrozen beef, but beef frozen at –114° F. was about 28 per cent more tender. The usual freezing temperatures for meat at present are from 0 to –10° F. Microscopic studies of frozen meat show that the tenderizing effect of freezing depends on how and where ice crystals form in the meat. In slow freezing, large crystals form between the muscle fibers bending the fibers out of shape but not breaking them. In fast freezing, at –10° F. or below, smaller crystals form within the fibers because there is no time for water to be drawn out. In forming, the crystals burst the fibers, thus tenderizing the meat. *Practical Home Economics 24,* 178 (1946).

SUNSHINE AND VITAMINS — Sunshine on growing fruits and vegetables has been found to exert an appreciable influence on the vitamin content of the mature product. Indications thus far seem to be that citrus fruits grown on the sunny side of the tree are richer in vitamin C than those grown on the shady side. The same has been found to be true for tomatoes, turnip greens and green beans. Vitamin A content, on the other hand, seems to increase in the shade. For example, in one study, tangerines contained to have 25 per cent more carotene when grown in the shade than those grown in the sun. *American Cookery 51,* 5 (1946).

PROTEIN DIGESTION — According to recently reported experiments, it has been postulated that the nutritive value of proteins may be considerably influenced by the

rate at which they are digested and their component amino acids are liberated. The value of a food as a source of protein is usually based on (1) its protein content in relation to total solids, (2) the essential amino acid composition of the protein and (3) the availability of these essential amino acids to the body tissues. Studies with hydrolyzed proteins have established the fact that the omission of a single essential amino acid from an otherwise adequate mixture results in poor utilization of the absorbed amino acids. If the missing amino acid is injected separately eight hours after the basic mixture is administered, it is too late to improve the retention of amino acid nitrogen. It is suggested, therefore, that for optimum utilization of food proteins all essential amino acids must not only be available for absorption, but must also be liberated during digestion in the body at rates permitting mutual supplementation. This theory has received experimental support in several preliminary studies with soy proteins. This hypothesis may have some significance in establishing the possible superiority of protein hydrolysates over the original intact proteins in that in hydrolysates the amino acids are liberated very rapidly in the body and would therefore be available for absorption almost as a group. *Science 103,* 325 (1946).